STUDY OF TROPONIN WITH CLEAVABLE PROTEIN CROSSLINKERS

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1. Introduction

Troponin is the protein associated with actin and tropomyosin in the thin filaments of vertebrate striated muscle which binds calcium and regulates muscle contraction (actin–myosin interaction) [1]. Troponin consists of one mole each of the three different components: troponin-T (37 000 molecular weight in rabbit) which binds to tropomyosin, troponin-I (24 000) which inhibits actin–myosin interaction in the presence and absence of calcium, and troponin-C (17 800) which binds calcium and interacts with troponin-I and T [2,3]. The topology of the isolated troponin complex containing these three components has been studied using the dimethylimidoester protein cross-linking reagents which covalently react with the ϵ-amino group of lysine [4]. Since the cross-linking with dimethylimidoesters is not easily reversible, we felt study of troponin cross-linked with the cleavable cross-linkers tartryldiazide (0.6 nm, TDA) and tartryl-di(glycylhydrazide) (1.3 nm, TDGA) would allow unequivocal identification of all the cross-linked products. These reagents are quantitatively cleaved by mild treatment with periodate and have been used for analysis of the arrangement of proteins in ribosomes [5] and adenovirions [6]. In the present study, when troponin is cross-linked with TDA and TDGA, the major products are the troponin-T+I+C and troponin-T+I complexes. In addition, the results show that small amounts of troponin-T+C and troponin-I+C complexes are detectable which could not be easily identified using the dimethylimidoesters. These results confirm that all three components are close to each other in the isolated troponin complex.

2. Materials and methods

Troponin was prepared from the back and thigh muscles of New Zealand rabbits according to Greaser and Gergely [2], was further purified on SP Sephadex C-50 [7], and was stored frozen at -20 °C.

Tartryl-diazide (TDA) and tartryl-di(glycylhydrazide) (TDGA) were synthesized as previously described [5].

Symmetrical two-dimensional sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis was carried out as described previously [5]. The cross-linked protein was electrophoresed on a 10% acrylamide slab gel. The strip containing the protein was incubated for 5 hours in 20 mM triethanolamine-HCl, pH 7.5, 0.1% SDS and then for 5 hours in the same buffer plus 10 mM $NaIO_4$ to cleave the cross-linker. The second dimension of electrophoresis was then carried out in the same conditions as the first dimension. The gels were fixed and stained in 0.25% Coomassie Brilliant Blue, 45% methanol, 10% acetic acid, and destained in 30% methanol, 10% acetic acid.

3. Results and discussion

When troponin is cross-linked with TDA or TDGA, the molecular weights of the two major cross-linked products are the same as those in troponin cross-

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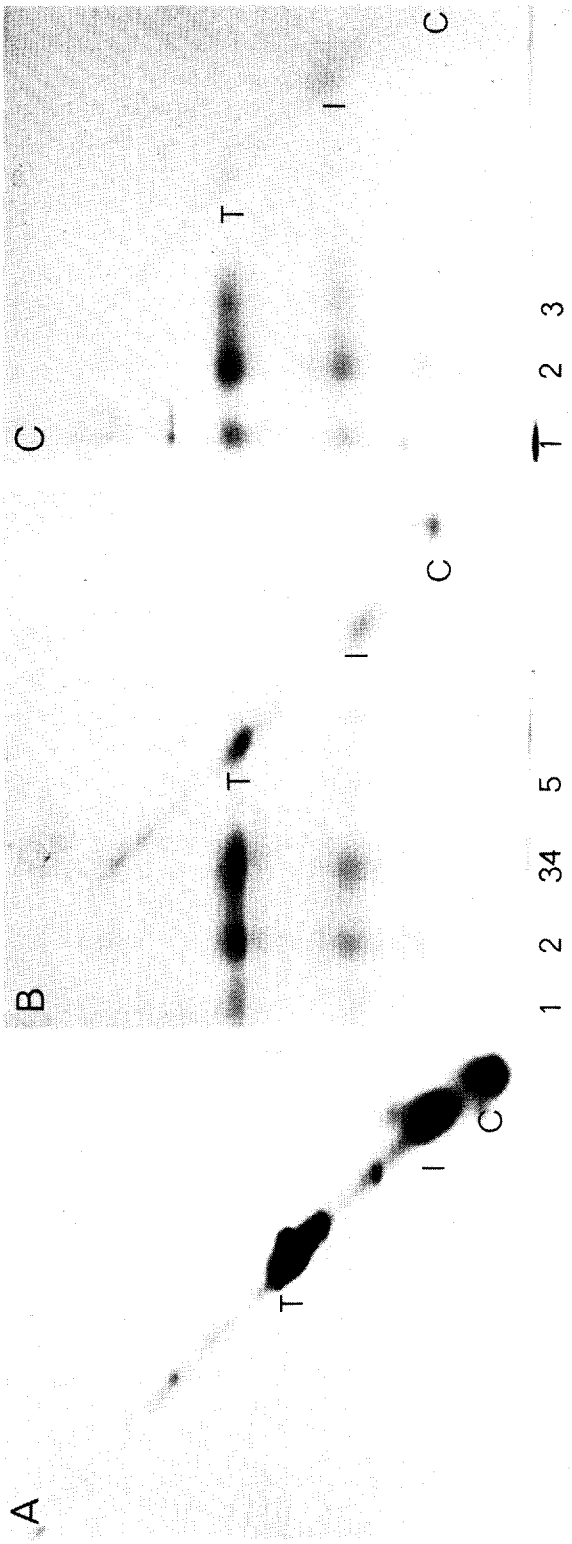

Fig. 2. Two-dimensional SDS acrylamide slab gels (10%) stained in Coomassie blue of A, control troponin; B, troponin cross-linked with TDA; C, troponin cross-linked with TDGA. Troponin in 50 mM triethanolamine–HCl, pH 8.5 60 mM KCl, 0.5 mM dithiothreitol, was sedimented for 4 hr at 80 000 *g* to remove aggregated material. Reagent was added to 10 mM and the reaction was carried out in the above buffer at 1 mg/ml protein for 30 min at room temperature (21 °C). The reaction was terminated by addition of methylamine–HCl to 50 mM and dialysis against 60 mM Tris–HCl, pH 7.2, 0.1% SDS. 75 μg protein were applied to each gel. The migration of troponin-C is sometimes anomalous in the two-dimensional gels. Figs.2B and 2C are from the same slab gel which was run at a different time than fig.2A.

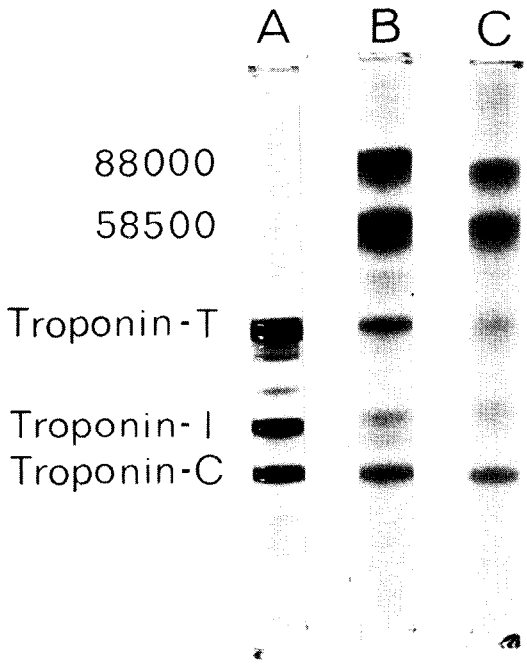

Fig.1. SDS acrylamide disc gels (10%) stained in Coomassie blue of A, control troponin; B, troponin cross-linked with dimethylsuberimidate [4]; C, troponin cross-linked with TDA. 30-35 μg protein were applied to each gel.

linked with dimethylsuberimidate: 88 000 and 58 500 (fig.1) [4]. In order to determine the composition of these cross-linked complexes, control and cross-linked troponin were subjected to a two-dimensional SDS polyacrylamide gel electrophoresis in which the protein cross-links were cleaved between dimensions (fig.2). Fig.2A is uncross-linked troponin. Uncross-linked proteins migrate the same distance in both dimensions and are seen on the diagonal after the second dimension. Figs.2B and 2C are troponin which has been cross-linked with TDA and TDGA, respectively. Protein which was cross-linked runs off the diagonal in the second dimension after cleavage of the cross-links. In both gels some aggregated material of high molecular weight is present (1). The major products of cross-linking are the 88 000 band (2) which is composed of troponin-T+I+C and the 58 500 band (3) which is cleaved into troponin-T+I. There are minor bands running faster than 58 500 composed of troponin-T+C (54 000, band 4) and troponin-I+C (45 000, band 5) which are faintly visible in original photographs of these gels. The TDA (0.6 nm) and TDGA (1.3 nm) give similar results except that with the longer cross-linker most of the protein is found in bands 2 and 3.

The appearance of all three possible combinations of cross-linked dimers (troponin-T+I, I+C, and T+C) as well as the ternary troponin complex (troponin-T+I+C) indicates that the three components are within 0.6 nm of each other in the isolated troponin complex. This result eliminates an arrangement in which two of the components are at a distance from each other on opposite sides of the third component.

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